OTITIS MEDIA IN NONSYNDROMIC OROFACIAL CLEFT FAMILIES

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ABSTRACT

Background: Otitis media (OM) is one of the most common infections diagnosed in young children around the world. Approximately $4.0 billion is spent annually on healthcare costs related to OM. Studies have shown OM to be highly prevalent (near 100%) in individuals with orofacial clefts (OC). OCs are the most common craniofacial birth defect in the world with approximately one in 500 to 1000 live births being affected. The medical costs for corrective surgeries alone pose a significant public health problem. These two conditions combined create a significant burden on the health, quality of life, and socioeconomic well-being of those affected and their family members.

The principal aim of this study is to investigate the frequency of OM in unaffected first-degree relatives of affected cleft individuals compared to controls, and to investigate and compare the reported rate of OM in individuals affected with different types of clefts.

Methods: The OC study of cleft lip and palate demographics and medical history questionnaire from the University of Pittsburgh 2009 was used to collect data for previous studies at 6 different study locations. Information collected via surveys was statistically analyzed using chi-squared analysis. Multiple comparisons were made between affected probands depending on cleft types, unaffected first-degree relatives, and controls.

Results: The likelihood of having chronic OM was increased in those with palatal involvement 2-fold (RR = 2.28, 95% CI = 2.02-2.57) over the cleft lip population. No statistically significant pattern was found in the siblings and parents of probands with different cleft forms. All cleft forms demonstrated a higher frequency of OM over the control population. Interestingly, the cleft lip population also demonstrated a 3-fold (OR = 3.09, 95% CI = 1.95-4.91) increased risk of OM over the control population, when previous studies provided evidence for no increase in this population. Siblings and parents failed to demonstrate statistically significant increases in OM compared to controls, and demonstrated a significantly reduced rate of OM than their affected relatives.

Conclusion: This study confirms that the NSOC population has a higher prevalence of OM than in the general population, with an increase in prevalence of OM in the CL population not described in historical research. There is no increase in prevalence of OM in the first-degree relatives of the cleft population.

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PREFACE

I would like to thank my thesis advisor, Seth, for developing this project and allowing me to take the first steps with this particular topic. I would also like to thank my advisors, Robin and Betsy, for guiding me through graduate school and helping me find myself along the way, I will always be in your debt. To my friends and family who supported me no matter what path I elected to take in life, I love you all and thank you. Finally, to my thesis committee, thank you for all of your support and suggestions throughout the process, I could not have done this without you.

1.0 INTRODUCTION

Approximately one in every 500 to 1000 live births is affected with an orofacial cleft (OC), making OC's a relatively common birth defect (Fogh-Andersen 1942; Woolf et al. 1963; 1964; Wyszynski et al. 1996; Croen et al. 1998; Weinberg, 2007a; 2007b; Dixon et al. 2011; Kohli et al. 2011; Yaqoob et al. 2013**)**. Worldwide, orofacial clefts are the most common craniofacial birth defect. The significant number of affected individuals as well as the substantial cost to repair OCs represents a major public health problem. The extensive surgeries required to repair the cleft place a substantial financial burden on the health care system (Weinberg, 2007a**)**. Multiple surgeries are required depending on the severity of the cleft and the extent of the craniofacial abnormalities. The surgeries are expensive, painful, time consuming, and emotionally draining for families and have the potential to create rifts within the family unit. Additionally, these children are susceptible to having feeding complications, require hearing and speech therapy, orthodontic treatment, otolaryngology treatment, psychosocial counseling, and treatment for recurring ear infections (Nemana et al. 1992; Neiswanger et al. 2002; Weinberg, 2006; 2007a; 2007b; Mossey et al. 2009; May, 2011**)**. Neonates with an OC have increased infant mortality and morbidity compared to unaffected children, especially for those in developing countries with limited care options for individuals with an OC (Dixon et al. 2011**)**.

The vast majority of OCs are isolated and are not present as part of a syndrome. Non-syndromic orofacial clefts (NSOC) account for approximately 75% of affected cases, while

approximately 25% are considered syndromic (Weinberg, 2007a**)**. While syndromic cleft cases are typically due to specific mutations or chromosomal abnormalities, identifying the genetic basis for NSOCs has been much more difficult due to the complex etiology (Woolf et al. 1963; Fraser 1970; Spirtz, 2001; Wyszynski et al. 1996; Weinberg, 2007a; Mossey, et al. 2009; Dixon et al. 2011; Kohli et al. 2012; Shkoukani et al, 2013; Yaqoob et al. 2013**)**. For families with an affected child, the chance of recurrence is often a major concern. Doctors and genetic counselors quote an empirical recurrence risk depending on the type of cleft and the family history. However, research has shown that the general population recurrence risk may not apply to all families (Weinberg, 2007a**)**. For some families a more accurate risk number can be determined based on specific phenotypic craniofacial abnormalities shared within families. Previous studies suggest that those with an NSOC and their unaffected relatives have related physical characteristics, which differ from the general population (Weinberg, et al. 2006; 2007a; 2007b; Marazita, 2012**)**.

OCs – especially those where the hard palate is affected, have a much greater risk of chronic otitis media (Paradise, 1969; Bluestone, 1971; 1972a; 1972b; 1975; 2004; Doyle et al. 1980; Shibahara et al. 1988Matsune et al. 1991a; 1991b; Daly et al. 2000; Sheahan et al. 2003; Lieberthal, 2006; Flynn et al. 2009; Sheer et al. 2010;**)**. Otitis media is defined as an infection in the middle ear and is a common occurrence in the general population. The majority of children have at least one episode of otitis media, with 50-85% of children affected before the age of three (Doyle et al. 1980; Daly et al. 2000; Flynn et al. 2009**)**. Approximately 10-20% of children under the age of one have recurrent otitis media, defined as three or more episodes, and nearly 40% of older children have six or more otitis media episodes in their lifetime (Sheahan, 2003**)**. With over 2.2 million cases of otitis media diagnosed each year in the United States and costing $4.0 billion

annually, otitis media is a major public health concern (Kemaloglu et al. 2000; Sheahan, 2003**)**. While children without OCs may require eustachian tube placement if the infection persists or occurs multiple times in a short period of time, nearly all children with isolated cleft palate (CP) have chronic otitis media and require eustachian tube placement. Children without a surgical repair of the palate can develop otitis media well into adulthood. Once a child has a repaired palate, their risk of developing chronic otitis media drops to that of the general population (Paradise, 1969; Bluestone, 1971; 1972a; 1972b; 1975; 2004; Doyle et al. 1980; Shibahara et al. 1988; Matsune et al. 1991a; 1991b; Daly et al. 2000; Sheahan et al. 2003; Lieberthal, 2006; Flynn et al. 2009; Sheer et al. 2010**)**.

The high incidence of otitis media in those affected with OCs can be explained by the anatomical disruption of the palatal shelves, which subsequently disrupts the position and orientation of the eustachian tubes thereby inhibiting proper drainage (Bluestone, 1971; 1975; Shibahara et al. 1988; Siegel et al. 1988; Sadler-Kimes et al. 1989; Takasaki et al. 2000; Sheahan et al. 2003; Bluestone, 2004**)**. Although a visible cleft may not be present, there is evidence that the palatal configuration in the parents and siblings of OC cases may also be abnormal. Such minor abnormalities are hypothesized to represent a sub-clinical phenotypic manifestation of an underlying genetic predisposition (Allen et al. 2014**)**. Because the palate may be abnormal, these unaffected relatives may be at elevated risk for eustachian tube dysfunction. To date, however, there are no data on the incidence of otitis media in the ostensibly unaffected family members of individuals with OCs. The present study will explore the occurrence of otitis media in a sample of NSOC families, focusing on both affected individuals with different types of clefts and their unaffected parents and siblings.

2.0 OROFACIAL CLEFTING

2.1 CLASSIFICATION AND PHENOTYPIC VARIABILITY

The classification of OCs has been proposed in a variety of different ways. There are several classification schemes based on anatomical location (Fogh-Andersen, 1942; Millard, 1976; Weinberg, 2007a). Clefts can be limited to the primary palate, which includes the lip and alveolus (CL or CL/A). There can also be clefts involving both the primary and secondary palate (CLP) and clefts involving only structures posterior to the incisive suture, which includes the soft palate (CP). When any form of clefting involves the primary palate, it can be either unilateral or bilateral.

Clefts can also be classified according to etiology. OCs can be part of a syndrome caused by single-gene mutations, chromosomal abnormalities, or teratogen exposures. However, the majority of OCs are nonsyndromic; they do not occur as part of a recognized syndrome and are present in the absence of additional malformations. NSOCs do not follow a simple (Mendelian) inheritance pattern and are considered complex genetic traits. Based on recurrence data, nonsyndromic CL and CLP are typically considered etiologically similar and part of the same phenotypic spectrum; together they are referred to as cleft lip with or without cleft palate (CL/P). Nonsyndromic CP is considered etiologically distinct. Murray (2002) reports that over 70% of all

CL/P cases are nonsyndromic, while approximately 50% of CP cases are considered nonsyndromic. This investigation focuses exclusively on nonsyndromic clefting (CL, CP, CLP).

2.2 EMBRYOLOGY

Facial development occurs between the fourth and eighth week post-conception (Yoon, 2000; Senders, 2003; Jiang, 2006). During the fourth week of development, there are several distinct facial prominences surrounding the primitive oral cavity. At the end of the fourth week of development two ectodermal thickenings (nasal placodes) appear on the frontonasal process, these are the precursors of the olfactory epithelium. During the fifth week, the olfactory network continues to develop with lateral nasal and medial nasal swellings, which surround the nasal placodes on the frontonasal process. While the lateral and medial nasal swellings grow forward, the nasal placodes invaginate providing the first step in development of the nasal cavities. Concurrently, paired maxillary processes develop from the mandibular prominences enlarging and growing (ventrally and medially) to surround the future oral cavity (Figure 1). Growing rapidly, the maxillary processes meet with the lateral nasal process forming the nasal fin. The breakdown of the nasal fin is required for the two nasal prominences to fuse with the medial nasal prominences. Jiang (2006) suggests that the fusion process involves restricted apoptosis and/or epithelial-mesenchymal transformation. In the sixth week of development the medial nasal processes start to form a primitive nasal septum and primary palate. These structures eventually form into the philtrum and complete upper lip late in the seventh week. The palatal shelves elevate and fuse forming the secondary palate as well during the seventh week. By the

beginning of the eighth week of development, the basic face is formed (Diewert et al. 1993a, 1993b, 2002; Diewert et al. 1993; Avery, 1994; Som et al. 2013).

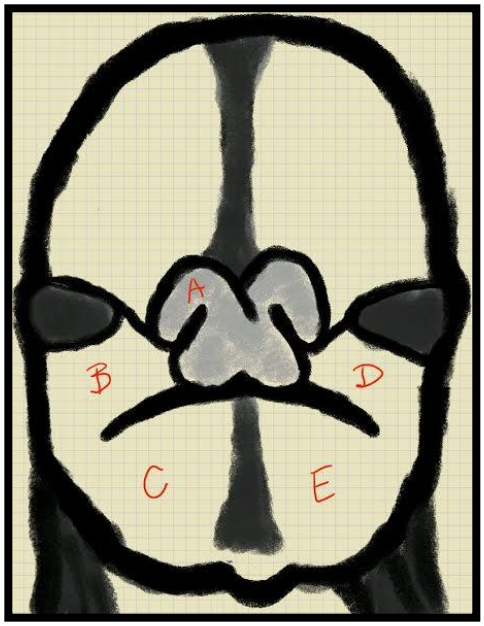

Figure 1: Face configuration on day 45 of development.

A. Frontonasal prominence; B. Right Maxillary Prominence;

C. Left maxillary Prominence; D. Left Mandibular Prominence

The skeletal and other connective tissues of the face are derived from neural crest derived mesenchyme (Grahm, 2003), while the facial musculature is derived from the cranial paraxial mesoderm (Noden et al. 2006). The epithelium of the face is derived from the surface covering the facial prominences, the ectoderm. Development of the face occurs when key genes are activated signaling the different tissues to interact (e.g. *Bmp4*) (Francis-West et al. 1998; Richman et al. 2003; Jiang, 2006; Parada et al. 2012). For the face to form properly, the process described above requires careful arrangement of multiple proteins to mediate the tasks of the bilateral symmetric cell migration, differentiation, growth, and apoptosis (May, 2011). An error in any part of this process can lead to partial or complete failure of the paired structures causing a cleft. There are a variety of cleft configurations described to date, ranging from a slight cleft of

the lip to a complete bilateral cleft of the lip and palate (Figure 2). Each cleft type is dependent on the protein error, which causes a specific part of the process to be affected (Cohen, 2006; Mossey et al. 2009; May, 2011).

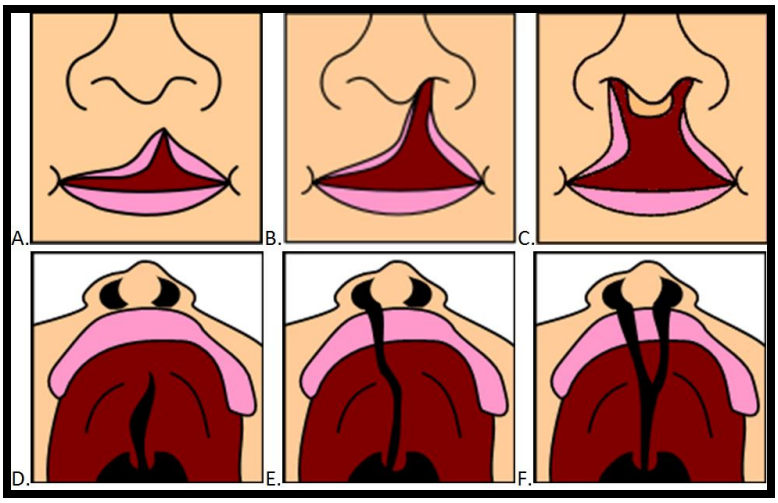

Figure 2: The Variety of Cleft Configurations (non-syndromic).

A. Incomplete Cleft Lip; B. Unilateral Complete Cleft Lip; C. Complete Bilateral Cleft Lip; D. Cleft Palate Only; E. Unilateral Cleft Lip and Palate; F. Bilateral Cleft Lip and Palate. *

2.3 EPIDEMIOLGY

Orofacial clefts are among the most common birth defects worldwide. Approximately one in every 500 to 1000 live births is affected (Fogh-Andersen, 1942; Woolf et al. 1963; 1964; Wyszynski et al., 1996; Croen et al. 1998; Weinberg, 2007a; 2007b; Dixon et al. 2011; Kohli et al. 2011; Yaqoob et al, 2013**).** In general, CL/P occurs more frequently than CP. The frequency of CLP also differs by sex with a 2:1 male to female ratio for clefts involving the lip and a 1:2

male to female ratio for cleft palate only. Furthermore, there is a 2:1 ratio of left to right sided clefts in unilateral cases (Dixon et al. 2011).

There is also ethnic variation in incidence. Croen et al. (1998) performed a study in California with 2,000 individuals with NSOCs of different ethnic populations demonstrating an incidence of 1.5-2 for every 1,000 live births. The incidence of clefting varied by population: White, Native American, African American, Hispanics, Japanese, and Chinese. The prevalence of CL/P was highest in Native Americans, then Whites, Japanese, Chinese, and African Americans. A similar ranking was found among the CP patients with Native American's again having the highest rate, followed by Whites, Hispanics, and African Americans. This study shows that although clefting is found worldwide, there are different ethnicities that show a higher incidence, which could suggest a genetic cause.

2.4 ETIOLOGY

CL/P and CP are complex genetic traits. They are often referred to as multifactorial, meaning there are multiple genetic and non-genetic (environmental) factors that can contribute to cleft susceptibility. Multifactorial diseases, or in this case birth defects, are complex due to the lack of a simple inheritance pattern. There is no defining Mendelian inheritance pattern (e.g., autosomal recessive or autosomal dominant) for complex disease traits, making it more difficult to predict the probability of passing on such a trait to the next generation. Multifactorial traits possess the same complexities as Mendelian diseases, such as: heterogeneity, variable expressivity, phenocopies, and reduced penetrance. Added to this are the complications of additive and/or multiplicative gene-gene and gene-environment interactions. Together, all of these factors make

the etiologies of complex traits difficult to determine, and since many diseases are complex, it is challenging to find causes for some of our most common health conditions (Weinberg, 2007a; Yaqoob et al. 2013).

The work by Fogh-Andersen (1942) thoroughly summarized hereditary cases of clefts in the 1920-1940s. Some researchers suggested an autosomal dominant inheritance, other suggested recessive; some researchers determined the trait was sex-linked, while others showed incomplete dominant inheritance patterns within families. Furthermore, by studying these cases, Fogh-Andersen was the first to distinguish between CL/P and CP, suggesting not only that most cases of CL/P were genetic, but that they were dominant sex-limiting to males. By the early 1960's, multifactorial patterns of inheritance were being developed and were immediately applied to CL/P (Woolf et al. 1963; 1964; Fraser, 1970; 1976; Carter et al. 1982; Hu et al. 1982; Weinberg, 2007a). By studying different models (e.g., multifactorial threshold theory, segregation analysis, goodness-to-fit approaches) it seemed that CL/P fit no distinct pattern, although there was evidence for every model, there was no exact fit (reviewed in Weinberg, 2007a). Families seemed to show a higher recurrence risk of producing a child with a cleft if a previous child was born with a cleft compared to the general population (Wyszynski et al. 1996). Therefore, familial patterns of recurrence were studied to determine the genetic basis of clefting (Weinberg, 2007a). These studies showed the recurrence risk for first-degree relatives is around 4% - 40 times greater than the general population - with the recurrence increasing as more individuals in a family are affected (Mitchell et al. 1993).

Many years of research have scrutinized CL/P families for a definitive genetic cause. In that time there are a number of different genes known to cause orofacial cleft syndromes, such as: Kabuki syndrome, Oral-facial-digital syndrome, chromosomal aberrations (such as

translocations) (Yagoob, 2013), Apert syndrome, Van der Woude syndrome, CL/P ectodermal dysplasia syndrome, Crouzon syndrome, Pierre Robin syndrome, Treacher Collins syndrome, (Kohli et al. 2012), and many more. However, a definitive genetic cause for the vast majority of NSOCs has not been identified. Candidate genes or loci have been the major focus for research in this discipline. Kohli et al. (2012), review the candidate genes found to date thought to be linked to NSOCs. Transforming growth factor-alpha (*TGFA*) is one such gene that has demonstrated a mixture of results, suggesting that certain mutations in this gene could be etiological to clefts, while others suggest that certain variants (TaqI C2 allele) together with maternal smoking and/or lack of prenatal vitamins in the first trimester increases the risk for a fetus to develop a cleft. The *Drosophila* msx homeobox homolog-1 (*MSX1*) gene was first found to cause autosomal dominant tooth agenesis and concurrent presence of CL/P. It has been suggested that *MSX1* mutations contribute to approximately 2% of all nonsyndromic CL/P (Jezewski et al. 2003). The enzyme 5,10-Methylenetetrahydrofolate reductase (MTHFR) is an important catalyst in the folate metabolism pathway. The *MTHFR* C677T single-nucleotide polymorphism (SNP) is a risk factor for neural tube defects and an increased risk for CLP (up to 10 times the general population risk). The gene transforming growth factor-beta-3 (*TGFB3*) is important in the adhesion of the opposing palatal shelves during face formation. The IVS5+104 A>G SNP was recently found to increase the risk of CL/P in the Korean population by as much as 16 times. There are many other candidate genes being researched, a review of all of these genes is beyond the scope of this paper.

In addition to genetic causes, there are wide varieties of environmental factors causing CL/P. Several of these are related to teratogenic and maternal health factors. A teratogen is defined as any substance that can cause malformations in a developing fetus (Wilson et al. 1977).

There are several drugs known to cause a teratogenic effect (causing a cleft) when taken during the first eight weeks of pregnancy, such as: hydantoin, sodium valproate, trimethadion, tranquilizers (Yaqoob et al. 2013), anti-convulsants (Abrishamchian et al. 1994), benzodiazepines (Dolovich et al. 1998), folate antagonists (Hernandez-Diaz et al. 2000), maternal smoking (Wyszynski et al. 1997), and alcohol (Munger et al. 1996; Kohliet al. 2012; Yaqoob et al. 2013). Each of these drugs has an effect on the developing fetus, typically in a dose-dependent manner (Shaw et al. 1999; Chung et al. 2000). Other teratogens such as toxins (organic solvents, and pesticides), as well as maternal infections associated with fever may be related to isolated clefts. Factors associated with maternal health have also been shown to play a role in cleft development (Hayes, 2002; Shashi et al. 2002; Weinberg, 2007a; Yaqoob et al. 2013). In animal studies, deficiencies in vitamins A, various B vitamins, and folic acid, have consistently been shown to result in OCs (Munger, 2002). Mothers suffering from diabetes mellitus or phenylketonuria are at higher risk of having a child with an OC (Weinberg, 2007a; Kohli et al. 2012; Yaqoob et al. 2013).

2.5 EXPANDED PHENOTYPE CONCEPT

OCs exhibit highly variable phenotypic expression. The clinical spectrum of clefting ranges from bilateral complete clefts extending all the way through the soft palate to microforms that appear as little more than a notch or scar on the upper lip (Weinberg et al. 2007b). In recent years, there has been renewed attention on how the OC phenotype is defined (Weinberg et al. 2006; Dixon et al., 2011). There is now a recognition that the phenotypic spectrum of clefts extends beyond “clinical” or “overt” manifestations. Subclinical expressions of the trait or of the same

underlying genetic risk factors have now been documented in cleft samples; these include altered brain morphology (Nopoulos et al. 2002; Weinberg et al. 2013), minor dental anomalies (Weinberg, et al. 2006; Shkoukani, et al. 2012), increased morphological asymmetry and/or altered laterality (Weinberg et al. 2006; Marazita, 2012), minor vertebral anomalies (Weinberg et al. 2006; Pisek et al. 2013), and dermatoglyphic abnormalities (Weinberg et al. 2006; Marazita, 2012). These expressions are typically subtle and may present with no functional deficit, eluding formal clinical recognition and characterization. Some are quantitative, requiring sophisticated phenotyping strategies. These additional phenotypic expressions can inform investigations into the etiology of OCs. For example, patterns of expression or co-expression of these subclinical traits may reveal distinct etiological subtypes of clefting.

Importantly, subclinical cleft phenotypes have also been documented in the unaffected (i.e., non-cleft) relatives of affected individuals (Weinberg et al. 2006). Beginning in the 1960's and stimulated by the emergence of multifactorial inheritance models, numerous reports have documented numerous subtle morphological differences statistically over-represented in the parents and siblings of cleft affected individuals compared to the general population. Some of these features are true micro-expressions of the trait, such as occult defects of the upper lip musculature only detectable on ultrasound (Weinberg et al. 2007b) or subtle speech abnormalities (Shkoukani et al. 2013). Others are best conceptualized as associated risk phenotypes, such as differences in craniofacial and dental morphology (reviewed in Weinberg et al. 2006). In either case, the presence of these traits in family members is believed to represent a mild expression of the underlying genetic susceptibility.

The expanded phenotype concept provides the conceptual framework for the present study. The current focus will be on the phenotype of otitis media – its co-occurrence with different forms of clefting and its presence in unaffected family members.

3.0 CLEFTING AND OTITIS MEDIA

3.1 OVERVIEW OF OTITIS MEDIA

It is estimated that the United States spends over $5 billion in healthcare for otitis media in children (Gould et al., 2010; Allen et al. 2014). Over 90% of children up to age 5 have experienced at least one occurrence of otitis media (Daly and Giebink, 2000; Bluestone, 2004; Lieberthal, 2006; Allen et al. 2014). However, otitis media may be under reported because the only way to establish a diagnosis with any certainty is using the pneumatic otoscope with visualization of the tympanic membrane with identification of a middle-ear effusion and inflammatory changes (Bluestone, 2004; Lieberthal, 2006). Those of a lower socioeconomic status may not have access to the healthcare system; therefore, the otitis media in these children may resolve on its own without medical intervention (Daly et al. 2000).

There are many different causes of otitis media infections. It is well known that the younger a child is, the higher the risk of developing an ear infection. The earlier one develops their first infection, the higher the risk of developing subsequent infections and chronic otitis media. As a child grows and develops, the angle and width of the eustachian tubes change, reducing the chances of developing otitis media [Figure 3] (Daly et al. 2000). Environmental factors also play a key role in the development of otitis media. Childcare attendance and exposure to young affected children (even siblings) greatly increases the risk for otitis media and

the requirement of tube placement. One study established a 2.5-fold risk of otitis media in children attending childcare outside the home (Uhari et al. 1996). Further environmental factors include protective effects of breast feeding when done for up to 3-6 months (with a 13% reduction in otitis media), and a detrimental effect of smoking with a 1.2 to 1.7 increase in incidence (Uhari et al. 1996; Daly et al. 2000; Bluestone, 2006). Furthermore, Daly et al. (2000), review a number of studies suggesting an increased otitis media risk in infants with very low birth weight, preterm birth, or intrauterine growth retardation. Structural abnormalities may also cause otitis media to occur. Since the growth and development of the anatomic region where the eustachian tube forms is associated with many other craniofacial abnormalities, it is evident that malformations in these craniofacial structures cause malformations of the eustachian tube (Kemaloglu et al. 2000).

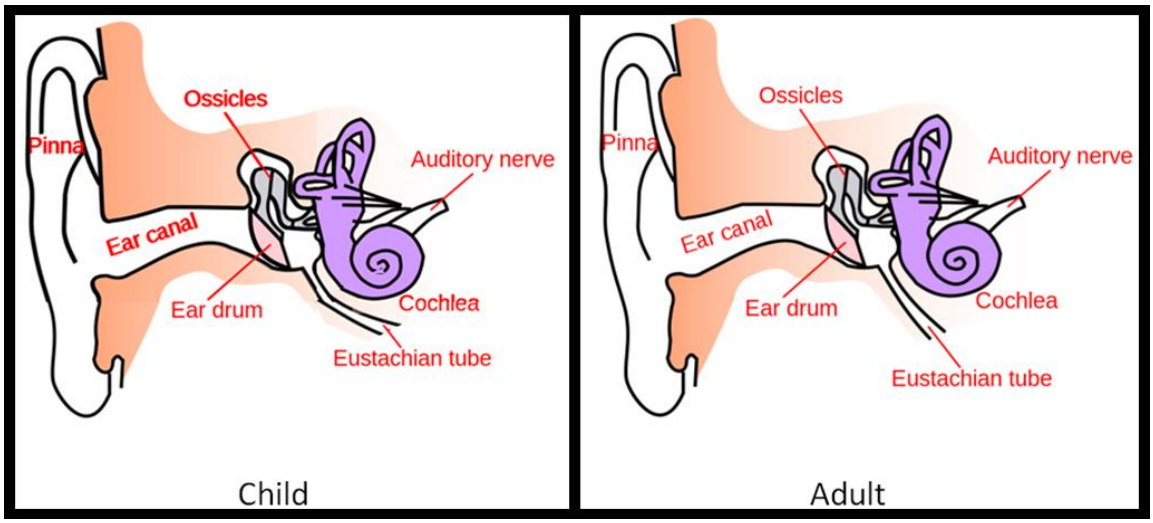

Figure 3: Eustachian tube differences in children and adults.

The adult eustachian tube has a steeper angle than the child's does. The steeper angle allows for easier drainage. The lack of drainage in a child's ear is a major cause for otitis media. **

Like NSOC, otitis media is considered a multifactorial disease. Allen et al. (2014) reviewed research for the evidence for a genetic contribution to otitis media. Family studies have shown heritability estimates as high as 74% (Allen et al. 2014). These studies provided the framework for genome-wide studies in search of specific genes/regions associated with otitis media. The majority of these genes are involved in the inflammatory and immune response. So far, tentative associations have been reported with *TLR4, MUC5B, SMAD2, SMAD4* (Allen et al. 2014). Family-based designs for linkage studies with siblings (pairs and larger sibships), nuclear families, and extended pedigrees have provided further insights (Allen et al. 2014).

Regardless of the cause, there are numerous treatment options available for those suffering from otitis media. Bluestone (2004), reviews, in depth, the different treatment options, which are beyond the scope of this thesis. Typically, treatment involves a course of systemic antibiotic (most commonly amoxicillin), which has shown to be effective not only as a treatment, but prophylactically as well. Surgical options have increased four-fold between 1970-1990 (Daly et al. 2000). Bluestone (2004) provides evidence for myringotomy and tympanostomy tube placement as providing the best treatment for those where surgical options are warranted. However, it seems that antibiotic in conjunction with surgical repair is the best option for most individuals.

3.2 PREVALENCE OF OTITIS MEDIA IN CLEFTING

Since 1969 when Paradise published his paper "*Diagnosis and management of ear disease in cleft palate infants,"* it has been accepted that otitis media is a common occurrence in cases of orofacial clefting (Paradise, 1969). He reported that cleft cases with a palatal involvement were

at much greater risk for developing otitis media compared to cases where the cleft involved only the lip. Paradise suggested that all infants with a cleft involving the secondary palate undergo routine otologic evaluation and have tubes placed as soon as possible to prevent otitis media. Many studies since Paradise have looked at the incidence of otitis media in relation to orofacial clefting. Sheahan et al. (2003) reported otitis media rate of 68% in CP patients, with a 45% recurrence rate, while there was an otitis media rate of 76% in CLP patients with a 46% recurrence risk. Suggesting a high rate of otitis media in patients affected with a cleft of the secondary palate; however, this rate is not nearly as high as Paradise's ~100% incidence of OM reported in 1969. Bluestone (1971), found that 78% of infants with CP required tubes for the treatment of their otitis media, with a higher rate of otitis media without tube placement, stating the probability of all infants with cleft palate having middle ear disease at, or shortly after, birth. He later states the "universal incidence" of a middle-ear effusion in patients with unrepaired cleft palate (Bluestone, 2004). Further evidence suggests a 74.7% prevalence of otitis media in the unilateral CLP population (Flynn et al. 2009). Though not all of these percentages agree, there is one evident fact – there is a high prevalence of otitis media in those affected with a cleft of the secondary palate.

Further studies on the topic by Bluestone (2004) suggest a reason for the high prevalence of otitis media in clefting. There are seven known abnormalities in the structure of the eustachian tube in cleft patients: shorter length of the tube, larger angle between cartilage and tensor veli palatine muscle, greater cartilage cell density, smaller ratio of lateral and medial laminae area of cartilage, less curvature of lumen, less elastin at hinge portion of cartilage, and less insertion ratio of tensor veli palatini to cartilage (Siegel et al. 1988; Shibahara et al. 1988; Sadler-Kimes et al. 1989; Sando and Takahashi, 1990; Matsune et al. 1991a; 1991b; Takasaki et al. 2000). These

differences help to illuminate the malformations of the eustachian tube caused by clefts, and the subsequent high rate of otitis media. The eustachian tube is part of a system of structures (palate, nasal cavities, nasopharynx, middle-ear, mastoid gas cell system). The function of the eustachian tube is to regulate the pressure in the middle ear, protect the middle ear from nasopharynx secretions, and drainage of middle-ear secretions into the nasopharynx. If the tube is too open or too closed, then there is abnormal pressure, which can cause an ear infection. Those with a cleft palate have a constricted eustachian tube, which impairs the normal drainage mechanism of the ear. If the tube cannot be opened to drain, then viruses or bacteria can remain within the tube causing major ear infections (Bluestone et al. 1972a; 1972b; 1975; 1980; 2004; Doyle et al. 1980a; 1980b; 1982; 1986).

Although the increased incidence of otitis media is well established in clefts involving the secondary palate (CP and CLP), far fewer studies have investigated clefts involving only the primary palate. Paradise (1969), initially reported a 96% rate for those with secondary palate involvement, while those with only CL reported a 17% rate of otitis media and controls having a 20% rate of otitis media. It is interesting that his results showed that the rate of otitis media was actually less than in the control population, but he did have a small sample size of 12 CL probands. Sheahan (2003) corroborates Paradise's data with a 16% rate of otitis media in their CL population compared to the 68% and 76% reported in their CP and CLP population, respectively. The highest rate of otitis media reported is Deelder et al. (2011); they found that 33% of CL cases in their study reported an episode of otitis media. These results suggest that, while the rate of infection is much lower than for clefts involving the secondary palate, there is at least a subset of CL cases where the palatal and/or eustachian tube anatomy may be compromised and are therefore susceptible to otitis media. Such findings could affect how CL

cases are classified, with implications for treatment, recurrence estimation, and gene identification.

3.3 OTITIS MEDIA AS A POTENTIAL MARKER FOR CLEFT RISK

The expanded OC phenotype concept was introduced in section 2.5. Using the same rationale, it may be plausible to consider isolated otitis media as a subclinical expression of orofacial clefting, particularly when it occurs in the non-cleft relatives of affected individuals. There is additional anatomical evidence to justify this hypothesis. As reported by Kemaloglu et al. (2000) the development of the eustachian tube is closely associated with the development of the cranial base and nasomaxillary complex, including the hard palate and associated structures. Numerous studies report mild craniofacial changes in the unaffected family members of cleft probands compared with controls (Weinberg et al. 2007a). Several studies have specifically noted subtle abnormalities in the palatal configuration and cranial base of these unaffected relatives (Weinberg et al. 2007a). Such changes are likely to impact the orientation, structure and subsequent function of the eustachian tubes. To date no study has investigated the occurrence of otitis media in the unaffected family members of cleft cases. Excessive otitis media in this population would provide the first line of evidence that the trait might be indicative of an underlying genetic risk in the absence of an overt cleft.

4.0 PURPOSE OF THE PRESENT STUDY

The aims of the present study are twofold: (1) to investigate the frequency with which otitis media occurs in the unaffected relatives (siblings and parents) from cleft families compared to both controls and affected cases; and (2) to investigate and compare the reported rate of otitis media in individuals affected with different types of clefts.

Our general working hypothesis is that unaffected relatives from NSOC families will display a higher frequency of otitis media compared to controls with no prior history of clefting; this is predicted for all cleft types. It is further predicted that relatives of individuals with clefts involving the secondary palate (CP and CLP) will exhibit a higher occurrence compared to the relatives of CL individuals. Moreover, within cleft-affected cases, it is predicted that the rate of otitis media in CL individuals will be higher than controls but lower than in CP or CLP cases.

5.0 MATERIALS AND METHODS

5.1 SAMPLE RECRUITMENT

Subjects for this study were recruited at six separate data collection sites; this included five US sites (Pittsburgh, Texas, Iowa, Colorado, and St Louis) and one international site (Budapest, Hungary). All subjects were recruited as part of a larger genetic and phenotypic study coordinated by the Center for Craniofacial and Dental Genetics at the University of Pittsburgh. Probands and families were identified through a combination of targeted advertisements, word-of-mouth, active recruitment at craniofacial centers, and various research registries and databases.

This study consisted of three groups based on the type of cleft present in the proband: cleft lip only, cleft palate only, and cleft lip and palate. Each group was comprised of probands (affected cases), the proband's unaffected parents, and the proband's unaffected sibling(s). Affected siblings and parents were excluded from this study. Families with a known syndromic form of clefting were also excluded. Unaffected controls with no prior history of clefting in their family were also recruited at several of the aforementioned sites (Pittsburgh, Iowa, St. Louis and Hungary).

5.2 DATA COLLECTION PROCEDURE

All recruitment and data collection procedures were conducted with the prior approval of the University of Pittsburgh Institutional Review Board (IRB) number 0405013 and additional local IRBs. Informed consent was obtained for each subject in the appropriate language prior to any data collection. Foreign and domestic data collection sites had slight differences in the questionnaire used. However, for the purposes of this study the questionnaire sections were designed in the same manner. The questionnaires were filled out by dedicated study staff at each site in an interview format. The answers were self-reported by family members or evident from the proband.

5.2.1 Cleft Status

Information on cleft status was obtained via a questionnaire. Proband cleft information was either self-evident or reported by family members. Medical history information such as specific cleft type in the proband and family members were verified at time of assessment as well. The LAHSHAL code was used to classify cleft phenotype (Kriens, 1989); this code was designed to provide researchers with a quick reference of cleft location (unilateral vs. bilateral; left vs. right) and type of defect (complete vs. incomplete; submucus, palatal, vs lip involvement).

5.2.2 Otitis Media Status

Otitis media information was self-reported by either the subject or the subject's immediate family member. A questionnaire was used to collect multiple points of medical information for

each subject. Otitis media questions included: if an ear infection had been diagnosed, age of ear infections, ear infection medications, if ear tubes had been placed, age first ear tubes placed (section to mark months or years for the age), and ear infections in family. Due to inconsistencies between the sites, exclusions had to be made as well as extrapolations of the data provided (see section 5.3 below).

5.2.3 Demographic Information

Basic demographic information such as age, sex, race, and ethnicity were obtained for each subject (proband and family members) at the time of assessment.

5.3 PARTICIPANT CRITERIA

Since this project was part of a much larger study, several considerations had to be taken into account in order to determine the final dataset and evaluate the relationship between orofacial clefting and otitis media. Several of these considerations are outlined below.

5.3.1 Inclusion/ Exclusion Criteria

In order for the proband, and therefore family, be included in this study, subjects: (1) had a personal history of non-syndromic (isolated) cleft lip, cleft palate, or cleft lip and palate, (2) had no history of facial or head trauma, (3) could not have a family history of syndromic orofacial clefts, and (4) had data for cleft type and ear infection. For siblings and parents, to be included in

this study: (1) they must be unaffected (i.e. no OC), (2) in the case of more than two siblings the unaffected male and female closest in age to the proband were selected for study, and (3) data for ear infection must be present.

5.3.2 Cleft Family Group Determination

Family data was sorted into the four groups listed above (CL, CP, CLP, Control) based on the clinical diagnosis of OC in the proband. In some cases family members of the proband (i.e.: parent and/or sibling) were affected with a different OC. In these cases, only the phenotype of the proband was taken into account to determine family group for this study. Therefore, if the proband had a CLP and a parent or sibling had a CP, the family group designation was CLP.

5.3.3 Extrapolations

Occasionally otitis media status for subjects needed to be extrapolated from additional questionnaire data. For example, a subject might not have indicated otitis media, but did indicate that they had tubes placed or provided evidence that they had taken medications for ear infections. In these cases, the subject was marked positive for otitis media.

5.4 SAMPLE CHARACTERISTICS

Otitis media information was available for a total of 2344 subjects from the six data collection sites. Data from 1751 cleft probands and their family members were included (Hungary, n = 672;

Texas n = 345; Iowa n = 555, Colorado, n = 62; Pittsburgh, n = 620; and St Louis, n = 91). A total of 593 control subjects were available for comparison. Controls were collected from the following sites: Hungary, n = 213; Iowa, n = 155; Pittsburgh, n = 188; and St. Louis, n = 37. Table 1 shows the complete sample broken down by group and sex.

Table 1: Sample size and mean age of groups represented in the present study.

	CP		CLP		CL		Controls	
	N	Age	N	Age	N	Age	N	Age
Affected Cases								
Combined	87	7.59	323	10.60	114	9.53	593	28.11
Male	45	7.42	204	9.95	63	7.91	245	26.55
Female	42	7.78	119	11.72	51	11.49	348	29.21
Siblings								
Combined	56	8.79	273	10.82	87	10.23		
Brothers	21	7.43	127	10.48	48	9.33		
Sisters	35	9.60	146	11.12	39	11.30		
Parents								
Combined	123	37.66	505	38.73	183	37.50		
Fathers	52	39.59	213	40.51	77	38.19		
Mothers	71	36.23	292	37.44	106	36.99		

5.5 DATA ANALYSIS

5.5.1 Statistical Analysis

Frequencies of otitis media were calculated and compared across group using Chi-square or Fisher's exact tests, as appropriate. Table 2 was created from initial count data and shows the frequency and proportion of subjects by group and sex with otitis media. A total of 81 univariate tests were run; adjusted for type 1 error due to multiple testing, the threshold for statistical significance was set at 0.0006.

Table 2: Frequency and proportion of reported otitis media by group.

	CP	CLP	CL	Controls
Affected Cases				
Combined	65/87 (74.7%)	230/323 (71.2%)	36/114 (31.6%)	77/593 (13.0%)
Male	33/45 (73.3%)	158/204 (77.5%)	21/63 (33.3%)	29/245 (11.8%)
Female	32/42 (79.2%)	72/119 (60.5%)	15/51 (29.4%)	48/348 (13.8%)
Siblings				
Combined	3/56 (5.4%)	41/273 (15%)	15/87 (17.2%)	
Brothers	1/21 (4.8%)	16/127 (12.6%)	12/48 (25.0%)	
Sisters	2/35 (5.7%)	25/146 (17.1%)	3/39 (7.7%)	
Parents				
Combined	8/123 (6.5%)	61/505 (12.1%)	25/183 (13.7%)	
Fathers	4/52 (7.7%)	15/213 (7.0%)	9/77 (11.7%)	
Mothers	4/71 (5.6%)	46/292 (15.8%)	16/106 (15.1%)	

6.0 RESULTS

6.1 COMPARING CLEFT CASES AND RELATIVES ACROSS CLEFT TYPES

Individuals with a cleft involving the secondary palate (CP, CLP, or both combined) demonstrated a significantly higher frequency of otitis media compared to cases with only primary palatal involvement (cleft lip probands) (Table 3, Table 4, and Table 5). The likelihood of having otitis media was increased more than 2-fold (RR = 2.28, 95% CI = 2.02-2.57) in cases affected probands with CP/CLP over CL individuals (Table 5). This pattern was not sex specific. In addition, CP and CLP cases showed no differences in the frequency of otitis media (Table 6).

In both the siblings and parents of probands with different forms of clefting, no clear pattern emerged. There was some evidence of increased otitis media in relatives of CL cases compared to relatives of CP cases (Table 3), but none of these differences reached statistical significance after adjusting for multiple testing. Likewise, no significant differences were noted when comparing the relatives (parents or siblings) between any of the cleft groups (Table 3, Table 4, Table 5 and Table 6).

Table 3: Comparing reported occurrence of otitis media in CP cases and family members to CL cases and family members.

	CP	CL	χ^2	P	RR	95% CI
Affected Cases						
Combined	65/87 (74.7%)	36/114 (31.6%)	36.72	< 0.001	2.37	1.59-3.52
Male	33/45 (73.3%)	21/63 (33.3%)	16.80	< 0.001	2.59	1.45-4.64
Female	32/42 (79.2%)	15/51 (29.4%)	20.16	< 0.001	2.20	1.53-3.16
Siblings						
Combined	3/56 (5.4%)	15/87 (17.2%)	4.37	0.037	0.31	0.11-0.89
Brothers	1/21 (4.8%)	12/48 (25.0%)	3.91	0.048	0.74	0.25-2.23
Sisters	2/35 (5.7%)	3/39 (7.7%)	0.11	0.740	0.19	0.15-0.24
Parents						
Combined	8/123 (6.5%)	25/183 (13.7%)	3.92	0.048	0.48	0.26-0.88
Fathers	4/52 (7.7%)	9/77 (11.7%)	0.55	0.458	0.37	0.15-0.91
Mothers	4/71 (5.6%)	16/106 (15.1%)	3.80	0.051	0.66	0.44-0.98

Table 4: Comparing reported occurrence of otitis media in CLP cases and family members to CL cases and family members.

	CLP	CL	χ^2	P	RR	95% CI
Affected Cases						
Combined	230/323 (71.2%)	36/114 (31.6%)	57.53	< 0.001	2.28	1.96-2.64
Male	158/204 (77.5%)	21/63 (33.3%)	29.37	< 0.001	2.06	1.67-2.54
Female	72/119 (60.5%)	15/51 (29.4%)	13.81	< 0.001	1.79	1.45-2.21
Siblings						
Combined	41/273 (15%)	15/87 (17.2%)	0.25	0.617	0.87	0.73-1.03
Brothers	16/127 (12.6%)	12/48 (25.0%)	3.99	0.046	2.23	1.91-2.60
Sisters	25/146 (17.1%)	3/39 (7.7%)	2.13	0.144	0.50	0.36-0.70
Parents						
Combined	61/505 (12.1%)	25/183 (13.7%)	0.31	0.578	0.88	0.77-1.02
Fathers	15/213 (7.0%)	9/77 (11.7%)	1.61	0.205	1.04	0.89-1.23
Mothers	46/292 (15.8%)	16/106 (15.1%)	0.03	0.863	0.60	0.44-0.83

Table 5: Comparing reported occurrence of otitis media in NSOC cases and family members with any secondary palate involvement to those with lip involvement only.

	CP and CLP	CL	χ^2	P	RR	95% CI
Affected Cases						
Combined	295/410 (72.0%)	36/114 (31.6%)	62.49	< 0.001	2.28	2.02-2.57
Male	191/249 (76.7%)	21/63 (33.3%)	43.43	< 0.001	2.30	1.94-2.73
Female	104/161 (64.6%)	15/51 (29.4%)	19.47	< 0.001	2.20	1.28-3.76
Siblings						
Combined	44/329 (13.4%)	15/87 (17.2%)	0.85	0.357	0.78	0.66-0.91
Brothers	17/148 (11.5%)	12/48 (25.0%)	5.25	0.022	0.46	0.34-0.63
Sisters	27/181 (14.9%)	3/39 (7.7%)	1.42	0.233	1.94	1.69-2.23
Parents						
Combined	69/628 (11.0%)	25/183 (13.7%)	0.99	0.320	0.80	0.71-0.91
Fathers	19/265 (7.2%)	9/77 (11.7%)	1.62	0.203	0.61	0.47-0.80
Mothers	50/363 (13.8%)	16/106 (15.1%)	0.12	0.729	0.91	0.79-1.06

Table 6: Comparing reported occurrence of otitis media in CLP cases and family members to CP cases and family members.

	CLP	CP	χ^2	P	RR	95% CI
Affected Cases						
Combined	230/323 (71.2%)	65/87 (74.7%)	0.28	0.597	0.96	0.86-1.07
Male	158/204 (77.5%)	33/45 (73.3%)	0.35	0.554	0.79	0.67-0.95
Female	72/119 (60.5%)	32/42 (79.2%)	3.34	0.068	2.32	1.89-2.86
Siblings						
Combined	41/273 (15%)	3/56 (5.4%)	3.74	0.053	2.80	2.54-3.09
Brothers	16/127 (12.6%)	1/21 (4.8%)	1.09	0.297	3.00	2.62-3.43
Sisters	25/146 (17.1%)	2/35 (5.7%)	2.40	0.089	2.65	2.30-3.04
Parents						
Combined	61/505 (12.1%)	8/123 (6.5%)	3.14	0.076	1.86	1.69-2.04
Fathers	15/213 (7.0%)	4/52 (7.7%)	0.03	0.863	2.80	2.53-3.09
Mothers	46/292 (15.8%)	4/71 (5.6%)	4.92	0.027	0.92	0.72-1.16

6.2 COMPARING CLEFT CASES TO CONTROLS

Individuals with a CP, CLP, and CL all demonstrated a higher frequency of otitis media (Table 7) compared with controls. Female probands with CL were the only group where the increase

over controls did not reach the threshold for statistical significance after bonferonni correction. The odds of having otitis media with a CP was increased nearly 20-fold (OR = 19.80; 95% CI = 11.54-34.0) for both sexes combined over the control population. Those with a CLP also displayed over 16-fold (OR = 16.57; 95% CI = 11.8-23.28) increased risk over controls and the CL population exhibited over a 3-fold (OR = 3.09; 95% CI = 1.95-4.91) increased risk.

Table 7: Comparing reported occurrence of otitis media in NSOC cases to controls.

	Cases	Controls	χ^2	P	OR	95% CI
CP						
Combined	65/87 (74.7%)	77/593 (13.0%)	174.97	< 0.001	19.80	11.54-34.0
Male	33/45 (73.3%)	29/245 (11.8%)	85.54	< 0.001	20.48	9.52-44.06
Female	32/42 (79.2%)	48/348 (13.8%)	89.49	< 0.001	20.00	9.24-43.31
CLP						
Combined	230/323 (71.2%)	77/593 (13.0%)	318.11	< 0.001	16.57	11.8-23.28
Male	158/204 (77.5%)	29/245 (11.8%)	197.20	< 0.001	25.58	19.14-42.5
Female	72/119 (60.5%)	48/348 (13.8%)	101.34	< 0.001	9.57	5.94-15.43
CL						
Combined	36/114 (31.6%)	77/593 (13.0%)	24.62	< 0.001	3.09	1.95-4.91
Male	21/63 (33.3%)	29/245 (11.8%)	17.03	< 0.001	3.72	1.94-7.15
Female	15/51 (29.4%)	48/348 (13.8%)	8.16	0.004	2.60	1.33-5.11

6.3 COMPARING UNAFFECTED RELATIVES TO CONTROLS

Siblings of probands affected with CP, CLP, and CL failed to demonstrate statistically significant increases in otitis media compared with controls; this was true regardless of sex (Table 8). The largest difference was between male siblings from CL families (25%) compared with male controls (11.8%). The pattern of results in unaffected parents was identical to siblings, with no statistically significant differences regardless of cleft family group or sex (Table 9). Of

all cleft types, siblings and parents of the CP group demonstrated the lowest frequency of otitis media, with lower rates than unaffected controls.

Table 8: Comparing reported occurrence of otitis media in the unaffected siblings of NSOC cases to controls.

	Siblings	Controls	χ^2	P	OR	95% CI
CP						
Combined	3/56 (5.4%)	77/593 (13.0%)	2.75	0.097	0.38	0.12-1.24
Male	1/21 (4.8%)	29/245 (11.8%)	0.97	0.325	0.37	0.05-2.88
Female	2/35 (5.7%)	48/348 (13.8%)	1.83	0.176	0.38	0.09-1.63
CLP						
Combined	41/273 (15.0%)	77/593 (13.0%)	0.66	0.417	1.18	0.79-1.78
Male	16/127 (12.6%)	29/245 (11.8%)	0.05	0.823	1.07	0.52-2.06
Female	25/146 (17.1%)	48/348 (13.8%)	0.91	0.340	1.29	0.73-2.10
CL						
Combined	15/87 (17.2%)	77/593 (13.0%)	1.17	0.279	1.40	0.76-2.56
Male	12/48 (25.0%)	29/245 (11.8%)	5.78	0.016	2.48	1.16-5.31
Female	3/39 (7.7%)	48/348 (13.8%)	1.14	0.286	0.52	0.15-1.76

Table 9: Comparing reported occurrence of otitis media in the unaffected parents of NSOC cases to controls.

	Parents	Controls	χ^2	P	OR	95% CI
CP						
Combined	8/123 (6.5%)	77/593 (13.0%)	4.09	0.043	0.47	0.22-0.99
Male	4/52 (7.7%)	29/245 (11.8%)	0.75	0.387	0.62	0.21-1.85
Female	4/71 (5.6%)	48/348 (13.8%)	3.61	0.057	0.37	0.13-1.07
CLP						
Combined	61/505 (12.1%)	77/593 (13.0%)	0.20	0.655	0.92	0.64-1.32
Male	15/213 (7.0%)	29/245 (11.8%)	3.02	0.082	0.56	0.31-1.14
Female	46/292 (15.8%)	48/348 (13.8%)	0.49	0.484	1.17	0.75-1.81
CL						
Combined	25/183 (13.7%)	77/593 (13.0%)	0.06	0.807	1.06	0.65-1.72
Male	9/77 (11.7%)	29/245 (11.8%)	0.00	1.000	0.99	0.44-2.19
Female	16/106 (15.1%)	48/348 (13.8%)	0.11	0.740	1.11	0.59-2.02

6.4 COMPARING UNAFFECTED RELATIVES TO CLEFT CASES

Compared with affected probands, both siblings and parents from CP and CLP families demonstrated significantly reduced rates of otitis media (Tables 10 and 11). The differences between probands and their relatives in CL families were more equivocal; the rate of otitis media was consistently higher in affected cases, but this difference was statistically significant only for parents (sexes combined).

Table 10: Comparing reported occurrence of otitis media in NSOC cases to their unaffected siblings.

	Cases	Siblings	χ^2	P	OR	95% CI
CP						
Combined	65/87 (74.7%)	3/56 (5.4%)	65.71	< 0.001	52.20	14.81-183.9
Male	33/45 (73.3%)	1/21 (4.8%)	26.95	< 0.001	55.00	6.64-455.60
Female	32/42 (79.2%)	2/35 (5.7%)	38.45	< 0.001	52.80	10.72-260.0
CLP						
Combined	230/323 (71.2%)	41/273 (15.0%)	188.39	< 0.001	13.99	9.29-21.09
Male	158/204 (77.5%)	16/127 (12.6%)	132.01	< 0.001	23.83	16.91-44.23
Female	72/119 (60.5%)	25/146 (17.1%)	53.17	< 0.001	7.41	4.21-13.06
CL						
Combined	36/114 (31.6%)	15/87 (17.2%)	5.36	0.021	2.22	1.12-4.38
Male	21/63 (33.3%)	12/48 (25.0%)	0.91	0.341	1.50	0.65-3.46
Female	15/51 (29.4%)	3/39 (7.7%)	6.52	0.011	5.00	1.33-18.77

Table 11: Comparing reported occurrence of otitis media in NSOC cases to their unaffected parents.

	Cases	Parents	χ^2	P	OR	95% CI
CP						
Combined	65/87 (74.7%)	8/123 (6.5%)	104.54	< 0.001	42.47	17.9-100.8
Male	33/45 (73.3%)	4/52 (7.7%)	44.05	< 0.001	33.00	9.79-111.3
Female	32/42 (79.2%)	4/71 (5.6%)	60.52	< 0.001	53.60	15.6-184.1
CLP						
Combined	230/323 (71.2%)	61/505 (12.1%)	302.17	< 0.001	18.00	12.56-25.8
Male	158/204 (77.5%)	15/213 (7.0%)	212.80	< 0.001	45.34	33.7-84.21
Female	72/119 (60.5%)	46/292 (15.8%)	82.72	< 0.001	8.19	5.05-13.29
CL						
Combined	36/114 (31.6%)	25/183 (13.7%)	13.82	< 0.001	2.92	1.64-5.20
Male	21/63 (33.3%)	9/77 (11.7%)	9.64	0.002	3.78	1.58-9.02
Female	15/51 (29.4%)	16/106 (15.1%)	4.45	0.035	2.34	1.05-5.23

7.0 DISCUSSION

The goals of this study were to investigate the frequency of otitis media in unaffected relatives in cleft families and compare the reported rates of otitis media in affected individuals with different cleft types. When comparing the cleft cases and relatives across cleft types (see section 6.1) there was an expected higher rate of otitis media in those affected with a cleft type involving the secondary palate (CP, CLP) compared to those with only primary palatal involvement (CL) regardless of sex. However, when comparing the parents and siblings of these individuals, the same pattern did not hold true. When comparing unaffected siblings and parents to controls, there was no statistically significant increase in otitis media. Interestingly, the siblings and parents of the CP probands showed the smallest rate of otitis media of any group. This could be due to the small sample size of the CP group. The CL and CLP groups each had a higher number of participants in the study than the CP group, which may be skewing the results. We predicted that family members of those affected with clefts involving the secondary palate would have a higher rate of otitis media than controls. Our failure to confirm this prediction could be due to aspects of study design. Parents may not remember having otitis media when they were young and therefore did not report it. There may also be a geographical bias, individuals who lived in more rural areas during their childhood may have never been properly diagnosed with otitis media.

The results obtained by this study demonstrate a clear association between NSOC and otitis media. These findings confirm that NSOC cases have a higher rate of otitis media than controls, but not the near 100% rate reported by Paradise (1969). Paradise had a select group of patients and was doing the confirmatory testing himself. He was able to diagnose the otitis media in the patients enrolled in his study. In contrast, in our study otitis media was self-reported, which may present some biases: participants may not remember having ear infections as a young child, may not feel it is important to report to the researcher, or may have never been diagnosed but still had an ear infection. Our results show up to a 79.2% otitis media rate in the CP female population, where the controls were 13.8%. All cases (CP, CLP, CL) showed a significant increase in otitis media over our control population except in the case of the female CL population.

Paradise reported that 17% of CL individuals had abnormal eardrums, compared to 20% of controls, suggesting that CL patients were at no greater risk for middle ear concerns than the general population (Paradise, 1969). Sheahan (2003) reported a 16% rate of recurrent otitis media in their CL population, compared to 68% and 76% reported in CP and CLP, respectively. However, a recent study by Deelder (2011) reported that 33% of CL probands in their sample had otitis media. This reported rate coincides with our study, with the combined sexes having a 31.6% rate of otitis media over the 13% rate in the control population. These two studies are suggestive of a defect in the eustachian tube in at least a subset of individuals affected with CL, raising several important questions. It suggests that some portion of CL probands may also have an underlying secondary palate abnormality. As further evidence, 28-40% of CL children were reported to have speech and language problems requiring therapy, which is significantly higher than the general population (Deelder et al. 2011). This has implications for children affected with

CL. Parents of CL children should be advised to have otological assessment at an early age, and to discuss speech and language therapy with the doctors by the age of two years (Deelder et al. 2011). Furthermore, the possible diagnostic uncertainty in cases of CL has potential implications for both genetic counseling and genetic studies of orofacial clefting. The presence of underlying secondary palate involvement in ostensibly CL children could alter recurrent risks. Further research into CL, palatal involvement, and otitis media should be done to better define recurrence risk estimation for genetic counselors. There is increasing evidence from genetic studies that CL and CLP are etiologically distinct defects (Harville et al. 2005; Rahimov et al. 2008). Consequently, the incorrect assignment of subjects to CL and CLP groups can reduce the accuracy and power of genetic studies of orofacial clefting by mixing cleft types with potentially distinctive etiologies.

7.1 LIMITATIONS

This study is characterized by several important limitations and biases. One limitation of was the relatively small sample size of some groups; in particular CP. One reason for this was that CL/P was the primary focus of the parent project from which this data were derived; CP subjects were often included out of convenience. All of the data regarding otitis media was self-reported, leading to several potential biases (discussed earlier). Further, in a number of cases otitis media had to be extrapolated from other types of data, which requires a judgment call. The researchers were required to choose siblings within a family for inclusion. Some families only had one child (the proband), while others had upwards of six children, some affected others not. The researcher's bias in choosing these subjects is noted and the inclusion of multiple family

members in multiple cases and no family members in other cases raises the possibility that some other factor present in these families are influencing the otitis media identified in multiple members.

7.2 FUTURE STUDIES

Future lines of study are required before this data can be submitted to the scientific community for review. An expanded population of cases, controls, siblings, and parents are essential to determine the type of cleft most related to otitis media. An expanded protocol for future studies in precise questioning regarding otitis media, the number of occurrences for each individual, the age of each occurrence, and eustachian tube placement is required as well. Medical records may be required to determine the extent of otitis media in participants and their families. Research should combine data from the Departments of Otolaryngology and Pediatrics at Children's Hospital of Pittsburgh, and the University of Pittsburgh Cleft Palate Center. Furthermore, age-of-onset related data were partially available for this study but were excluded due to insufficient information and confusing answers. A future study could concentrate on the age of family members at the time of first otitis media occurrence to determine if there are any age effects across the different cleft phenotypes. This information would provide guidance for craniofacial teams, audiology specialists, and genetic counselors in the future in order to counsel families with the best information possible and give them the most accurate risk assessment of phenotypes in individual family members.

7.3 CONCLUSIONS

In conclusion, the present study confirms that the NSOC population has a higher prevalence of otitis media than in the general population, but does not show a sex-effect or a higher prevalence in family members of those affected. Interestingly, this study does show an increase in the prevalence of otitis media in the CL population, which has only been shown in a select number of research studies and may be indicative of subtle anatomical disruptions of secondary palate and/or associate structures.

BIBLIOGRAPHY

Abrishamchian, A.R. et al. (1994) "The contribution of maternal epilepsy and its treatment to the etiology of oral clefts: a population based case-control study. Genet Epidemiol. 11: 343-351.

Allen, E.K. et al. (2014) "Genetic contributors to otitis media: Agnostic discovery approaches." Curr Allergy Asthma Rep. 14: 411-417.

Avery, J.K. (1994) "Development of the branchial arches, face, and palate. In: Avery JK (ed) *Oral Development and Histology*.Thieme Medical Publishers, Inc., New York, pp 20-41.

Bear, J.C. (1976) "A genetic study of facial clefting in Northern England." Clin Genet. 9: 277-284.

Bluestone, C.D. (1971) "Eustachian tube obstruction in the infant with cleft palate." Ann Otol Rhino Larygol. 80(Suppl. 2): 1-30.

Bluestone, C.D. et al. (1972a) "Physiology of the Eustachian tube in the pathogenesis and management of middle-ear effusions." Laryngoscope. 82: 1654-1670.

Bluestone, C.D. et al. (1972b) "Roentgenographic evaluation of Eustachian tube function in infants with cleft and normal palates." Cleft Palate J. 9: 93-100.

Bluestone, C.D. et al. (1975) "Eustachian tube ventilator function in relation to cleft palate. Ann Otol Rhino Larygol. 84: 333-338.

Bluestone, C.D. (2004) "Studies in otitis Media: Children's Hospital of Pittsburgh – University of Pittsburgh Progress Report – 2004." Laryngoscope. 114(Suppl.105): 1-26.

Carter, C.O. et al (1982) "A three generation family study of cleft lip with or without cleft palate. J Med Genet. 19: 246-261.

Chen, Y-W. et al. (2012) "Is Otitis Media with Effusion Almost Always Accompanying Cleft Palate in Children?: The Experience of 319 Asian Patients." Laryngoscope. 122: 220-224.

Chung, K.C. et al. (2000) "Maternal cigarette smoking during pregnancy and the risk of having a child with cleft lip/palate." Plast Reconstr Surg. 105: 485-491.

Cohen, M.M.J. (2006) *Perspectives on the face.* Oxford University Press, New York.

Croen, L.A. et al. (1998) "Racial and Ethnic Variations in the Prevalence of Orofacial Clefts in California, 1983-1992." Am J Med Gen. 79: 42-47.

Daly, K.A. and Giebink, G.S. (2000) "Clinical epidemiology of otitis media." Pediatr Infect Dis J. 19: S31-6.

Deelder, J.D. et al. (2011) "Is an isolated cleft lip an isolated anomaly?" J Plast Reconstr Aesthet Surg. 64(6):754-8.

Diewert, V.M. and Lozanoff, S. (1993a) "A morphometric analysis of human embryonic craniofacial growth in the median plane during primary palate formation." J Craniofac Genet Dev Biol. 13: 162-183.

Diewert, V.M. and Lozanoff, S. (1993b) "Growth and morphogenesis of the human embryonic midface during primary palate formation analyzed in frontal sections." J Craniofac Genet Dev Biol. 13: 162-183.

Diewert, V.M. and Lozanoff, S. (2002) "Animal models of facial cleftin: experimental, congenital, and transgenic." In: Mooney, M.P. and Siegel M.I. (eds) *Understanding craniofacial anomalies: The etiopathogenesis of craniosynostoses and facial clefting.* Wiley-Liss, Inc., New York, pp 251-272.

Diewert, V.M. et al. (1993) "Computer reconstructions of human embryonic craniofacial morphology showing changes in relations between the face and brain during primary palate formation." J Craniofac Genet Dev Biol. 13: 193-201.

Dixon, M.J. et al. (2011) "Cleft lip and palate: Synthesizing genetic and environmental influences." Nat Rev Genet. 12(3): 167-178.

Dolovich, L.R. et al. (1998) "Benzodiazepine use in pregnancy and major malformations or oral cleft: meta-analysis of cohort and case-control studies." BMJ. 317: 839-843.

Doyle, W.J. et al. (1980a) "Eustachian tube function in cleft palate children. Ann Otol Rhinol Laryngol. 89(Suppl 68):34-40.

Doyle, W.J. et al. (1980b)"Nonhuman primate model of cleft palate and its implications for middle ear pathology." Ann Otol Rhinol Laryngol. 68(Suppl 68): 41-46.

Doyle, W.J. et al. (1982) "Anomalous middle ear gas absorption in a non-human primate model of cleft palate. Cleft Palate J. 19: 17-24.

Doyle, W.J. et al. (1986) "Effect of palatoplasty on the function of the Eustachian tube in children with cleft palate." Cleft Palate J. 23: 63-68.

Doyle, W.J. et al. (1980) "Eustachian tube function in cleft palate children." Ann Otol Rhinol Laryngol. 89(3 Pt 2): 34-40.

Flynn, T. et al. (2009) "The high prevalence of otitis media with effusion in children with cleft lip and palate as compared to children without clefts." Int J Ped Otol. 73: 1441-1446.

Fogh-Andersen, P. (1942) *Inheritance of Hare Lip and Cleft Palate.* Nyt Nordisk Forlag Arnold Busck, Copenhagen.

Francis-West, P.H. et al. (1998) "Signaling interactions during facial development." Mech Dev. 75: 3-28.

Fraser, F.C. (1970) "The genetics of cleft lip and cleft palate." Am J Hum Genet. 22: 336-352.

Fraser, F.C. (1976) "The multifactorial/threshold concept-uses and misuses." Teratology. 14: 267-280.

Gould, J.M. and Matz, P.S. (2010) "Otitis Media." Ped Rev Am Acad Pediatr. 31(3): 102-116.

Graham, A. (2003) "The neural crest." Curr Biol. 13: R381-R384.

Harris, E.F. (2002) "Dental development and anomalies in craniosynostoses and facial clefting." In: Mooney, M.P. and Siegel, M.I. (eds) *Understanding Craniofacial Anomalies: the Etiopathogenesis of Craniosynostoses and Facial Clefting.* Wiley-Liss, Inc., New York, pp 425-467.

Harville EW, Wilcox AJ, Lie RT, Vindenes H, Abyholm F. (2005) Cleft lip and palate versus cleft lip only: are they distinct defects? Am J Epidemiol. 162: 448-453.

Hayes, C. (2002) "Environmental risk factors and oral clefts." In: Wyszynski D.F. (ed) *Cleft Lip and Palate: From Origin to Treatment.* Oxford University Press, Oxford, pp159-169.

Hernandez-Diaz, S. et al. (2000) "Folic acid antagonists during pregnancy and the risk of birth defects." N Engl J Med. 343: 1608-1614.

Jezewski, P.A. et al. (2003) "Complete sequencing shows a role for *MSX1* in non-syndroic cleft lip and palate. J Med Genet. 40: 399-407.

Jiang, R. et al. (2006) "Development of the upper lip: morphogenetic and molecular mechanisms." Dev Dyn. 235: 1152-1166.

Kemaloglu, Y.K. et al. (2000) "Associations between the Eustachian tube and craniofacial Skeleton." Intl J Ped Otol. 53: 195-205.

Kohli, S.S. and Kohli, V.S. (2012) "A comprehensive review of the genetic basis of cleft lip and palate." J Oral Maxillofac Pathol. 16(1): 64-72.

Lieberthal, A.S. (2006) "Acute Otitis Media Guidelines: Review and Update." Curr Allergy and Asthma Reports. 6: 334-341.

Marazita, M.L. (2012) "The Evolution of Human Genetic Studies of Cleft Lip and Cleft Palate." Annu Rev Genomics Hum Genet. 13:263-283.

Matsune, S. et al. (1991a) "Abnormalities of lateral cartilaginous lamina and lumen of Eustachian tube in cases of cleft palate." Ann Otol Rhinol Laryngol. 100: 909-913.

Matsune, S. et al. (1991b) “Insertion of the tensor veli palatine muscle into the Eustachian tube cartilage in cleft palate cases.” Ann Otol Rhinol Laryngol. 99: 13-16.

May, M.A. (2011) “Olfactory Deficiets in Cleft Lip and Palate.” Graduate Thesis.

McIntyre, G.T. and Mossey, P.A. (2002) “The craniofacial morphology of the parents of children with orofacial clefting: a systematic review of cephalometric studies.” J Orthod. 29: 23-29.

Melnick, M. et al. (1980) “Cleft lip +/- cleft palate: an overview of the literature and an analysis of Danish cases born between 1941 and 1968.” Am J Med Gen. 6: 83-97.

Millard, D.R. (1976) *Cleft Craft: The Evolution of its Surgery.* Little, Brown, Boston.

Mitchell, L.E. and Risch, N. (1993) “Correlates of genetic risk for non-syndromic cleft lip with or without cleft palate.” Clin Gen. 43: 255-260.

Mossey, P.A. et al. (2009) “Cleft lip and palate.” Lancet 374(9703): 1733-1785.

Munger, R.G. (2002) “Maternal nutrition and oral clefts.” In Wyszynski D.F. (ed) *Cleft Lip and Palate: From Origin to Treatment.* Oxford University Press, Oxford, pp 170-192.

Munger, R.G. et al. (1996) “Maternal alcohol use and risk of orofacial cleft birth defects.” Teratology. 54: 27-33.

Murray, J.C. (2002) “Gene/environment causes of cleft lip and/or palate.” Clin Gen. 61:248-256.

Neiswanger, K. et al. (2002) “Cleft lip with or without cleft palate and dermatolglyphic asymmetry: evaluation of a Chinese population.” Orthod Craniofacial Res. 5: 140-146.

Nemana, L.J. et al. (1992) “Genetic analysis of cleft lip with or without cleft palate in Madras, India.” Am J Med Gen. 42: 5-9.

Noden, D.M. and Francis-West, P. (2006) “The differentiation and morphogenesis of craniofacial muscles.” Dev Dyn. 235: 1194-1218.

Nopoulous, P. et al. (2002) “Cognitive dysfunction in adult males with non-syndromic clefts of the lip and/or palate.” Neuropsychologia. 40: 2178-2184.

Parada, C. and Chai, Y. (2012) “Roles of BMP signaling pathway in lip and palate development.” Front Oral Biol. 16: 60-70.

Paradise, J.L. and Bluestone, C.D. (1969) “Diagnosis and management of Ear Disease in Cleft Palate Infants.” Ophth. & Otol. 73(4): 709-714.

Pisek, P. et al. (2013) “A comparison of cervical vertebral maturation assessment of skeletal growth stages with chronological age in Thai between cleft lip and palate and non-cleft patients.” J Med Assoc Thai. 96(Suppl 4):S9-18.

Rahimov F, Marazita ML, Visel A, Cooper ME, Hitchler MJ, Rubini M, Domann FE, Govil M, Christensen K, Bille C, Melbye M, Jugessur A, Lie RT, Wilcox AJ, Fitzpatrick DR,

Green ED, Mossey PA, Little J, Steegers-Theunissen RP, Pennacchio LA, Schutte BC, Murray JC. (2008) Disruption of an AP-2 alpha binding site in an IRF6 enhancer is associated with cleft lip. Nat Genet. 40: 1341-1347.

Richman, J.M. and Lee, S-H (2003) "About face: signals and genes controlling jaw patterning and identity in vertebrates." Bioessays. 25: 554-568.

Sadler-Kimes, D. et al. (1989) "Age-related morphologic differences in the components of the Eustachian tube/middle ear system." Ann Otol Rhinol Laryngol. 98: 854-858.

Sando, I. and Takahashi, H. (1990) "Otitis media in association with various congenital diseases." Ann Otol Rhinol Laryngol. 99: 13-16.

Senders, C.W. (2003) "Development of the Upper Lip." Arch Facial Plast Surg. 5: 16-25.

Shashi, V. and Hart, T.C. (2002) "Environmentla etiologies of orofacial clefting and craniosynostosis." In: Mooney, M.P. and Siegel, M.I. (ed) *Understanding Craniofacial Anomalies: The Etiopathogenesis of Craniosynostoses and Facial Clefting.* Wiley-Liss, Inc., New York, pp 163-205.

Shaw, G.M. and Lammer, E.J. (1999) "Maternal periconceptional alcohol consumption and risk for orofacial clefts." J Pediatr. 134: 298-303.

Sheahan, P. et al. (2003) "Incidence and outcome of middle ear disease in cleft lip and/or cleft palate." Intl J Ped Otol. 67: 785-793.

Sheer, F.J. et al. (2010) "Finite Element Analysis of Eustachian Tube Function in Cleft Palate Infants Based on Histological Reconstructions." Cleft Palate-Craniofacial J. 47(6): 600-610.

Shibahara, Y. et al. (1988) "Histopathologic study of Eustachian tube in cleft palate patients." Ann Otol Rhinol Laryngol. 97: 403-408.

Shkoukani, M.A. et al. (2013) "Cleft Lip – A Comprehensive Review." Front Pediatr. 1:53.

Siegel, M.I. et al. (1988) "ET cartilage shape as a factor in the epidemiology of otitis media. In: Lim D.J. et al. *Recent Advances in Otitis Media: proceedings of the Fourth International Symposium.* Hamilton, Ontario, Canada: BC Decker: 114-117.

Som, P.M. and Naidich, T.P. (2013) "Illustrated review of the embryology and development of the facial region, part 1: Early face and lateral nasal cavities." AJNR Am J Neuroradiol. 34(12): 2233-2240.

Spirtz, R.A. (2001) "The genetics and epigenetics of orofacial clefts." Curr Opin Ped. 13: 556-560.

Takasaki, K. et al. (2000) "Postnatal development of Eustachian tube cartilage: a study of normal and cleft palate cases." Int J Pediatr Otorhinolaryngol. 52: 31-36.

Uhari, M. et al. (1996) "A meta-analytic review of the risk factors for acute otitis media. Clin Infect Dis. 22: 1079-83.

Ward, R.E. et al. (2002) “Morphometric characteristics of subjects with oral facial clefts and their relatives.” In: Wyszynski, D.F. (ed) *Cleft Lip and Palate: From Origin to Treatment.* Oxford University Press, Oxford, pp 66-86.

Weinberg, S.M. et al. (2006) “The Pittsburgh Oral-Facial cleft Study: Expanding the Cleft Phenotype. Background and Justification.” Cleft Palate-Craniofacial J. 43(1): 7-20.

Weinberg, S.M. (2007a) “Three-Dimensional morphometric analysis of the craniofacial complex in the unaffected relatives of individuals with nonsyndromic orofacial clefts.” Doctoral Thesis.

Weinberg, S.M. et al. (2007b) “The use of ultrasound to visualize the upper lips of noncleft and repaired-cleft individuals.” Cleft Palate Craniofac J. 44(6):683-4.

Wilson, J.G. and Fraser, F.C. (1977) *Handbook of Teratology, Vols. 1-4.* Plenum Press, New York.

Woolf, C.M. et al. (1963) “A genetic study of cleft lip and palate in Utah.” Am J Hum Gen. 15: 209-215.

Woolf, C.M. et al. (1964) “Cleft lip and hereditary.” Plast Reconstr Surg. 34: 11-14.

Wyszynski, D.F. et al (1996) “Genetics of nonsyndromic oral clefts revisited.” Cleft Palate Craniofacial J 33: 406-417.

Yaqoob, M. et al. (2013) “Etiology and genetic factors in clefts of lip and/or palate reported at children’s hospital, Lahore, Pakistan.” Indian J Hum Genet. 19(2): 136-143.

Yoon, K.J. et al. (2000) “Development of the lip and palate in staged human embryos and early fetuses.” Yonsei Med J. 41: 477-484.

*Felsir. *Cleft Lip and Palate.* [Images]. Retrieved 27 Jan 2014 from http://en.wikipedia.org/wiki/Cleft_lip_and_palate

**Iain. *Eustachian Tube.* [Altered Image]. Retrieved 27 Jan 2014 from http://en.wikipedia.org/wiki/Eustachian_tube)